NOTES ON THE BIONOMICS OF *STEGOMYIA CALOPUS*, Meigen, IN BRAZIL

PART I

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The following work, which was carried out in Manáos, does not attempt to deal comprehensively with the bionomics of *Stegomyia calopus*, but is mainly concerned with various points which attracted attention while breeding these mosquitoes in the laboratory. It was undertaken chiefly owing to the noting of some slight differences in the bionomics of this mosquito, as compared with those observed in certain other countries.

The factors influencing the bionomics of *S. calopus* have been shown to be complex, and, owing to its sensitiveness to environmental conditions, it is doubtful if conclusions can be drawn from the comparison of experiments which have not been carried out under similar conditions of time, place, etc. The effects of many of these factors may be studied by comparison with controls, the only difference between the control and the subject of the experiment being the factor under investigation. Where applicable, this method was adopted in the following experiments.

METHODS

The adult mosquitoes were kept in wire-gauze cages, and fed on sugar solution. When eggs were required they were allowed to feed on human blood. In the experiments, eggs and larvae were kept in glass jars on a bench in the laboratory, and those used in any one experiment in which a comparison was to be made were kept together, so that the temperature and amount of light reaching each one were the same.

Except where otherwise stated, larvae which hatched out were removed every twenty-four hours. The eggs used in each experiment where comparisons are made were from the same batch of eggs, but not necessarily from the same adult, the eggs being mixed before distribution. They were always less than twenty-four hours old at the beginning of each experiment.

TEMPERATURE

The temperature range in Manáos throughout the year is small. According to official figures from 1902 to 1914, the average of the annual mean temperature was 28·2° C., the absolute maximum 38·6° C., and the absolute minimum 18·8° C. During the experiments the laboratory temperature varied between 33° C. and 24·5° C. The temperature of the water in the jars was always found to be within 2° C. of the atmospheric temperature, and was frequently the same. The daily range of the laboratory temperature was usually less than 6° C.

LAYING OF EGGS

Fielding (1919) and Bacot (1916) found that females preferred contaminated water to clear for laying eggs in. A few experiments were carried out to discover if this preference existed in Manáos.

Watch glasses containing the waters to be compared were placed in the breeding-cage, losses due to evaporation being replaced from time to time. The results, which are given in Table I, are similar to those of Fielding and Bacot.

TABLE I.

Laying of Eggs in Different Waters.

Duration of Experiment	Contents of Glass	No. of Eggs laid	Percentage of Total
72 days	Tap water	560	26
	River water	1590	74
32 days	Tap water	563	10·6
	River water	1682	31·5
	Water from cesspool	3092	57·9

Bacot states that it is misleading to say that the eggs are deposited on the surface of water, as in the great majority of cases they are to be found on the wet margins of the receptacle or other object, and that no instance of an egg being laid on a dry surface was observed.

In the following experiment it was found that in captivity the majority of eggs were laid on a damp surface, when available. A watch glass containing rain water and a piece of blotting paper floating on it, the areas of the blotting paper and the water surface exposed being approximately equal, was placed in the breeding-cage. The results are shown in Table II.

TABLE II.

The Numbers of Eggs laid on Water and on a Damp Surface.

Duration of Experiment	Eggs found		
	On Blotting Paper	On Water	On Dry Glass
44 days	1966	568	88

No mosquitoes were observed laying eggs on a dry surface, and those found dry were probably stranded by capillary attraction and left above water level by evaporation. This could not occur with the blotting paper, as it was floating. Fielding's findings in Queensland were similar.

So far as hatching was concerned, no difference was found between those laid on the moist blotting paper and those laid on water.

Wild *Stegomyia* have been observed on several occasions laying eggs in barrels standing in the enclosure behind the laboratory. The eggs were always laid on the wet sides of the barrel just above the water surface. In glass jars placed outside, eggs were laid on the water, usually near the sides, and many adhered to the jar as the water evaporated.

HATCHING OF EGGS

Difficulty was at first experienced in getting eggs which were left floating as laid, to hatch. An attempt was, therefore, made to discover the cause of this, and several factors were found to influence the hatching. Those investigated were, position of the eggs (floating or submerged), presence or absence of disturbance, presence or absence of food, and the nature of the water. Many other factors, such as bacterial action, formalin, temperature, humidity, drying, lysol, petroleum, soft soap emulsion, and soap solution, are stated to influence the hatching of the eggs.

Many experiments were carried out, all of which are not given below, but the following illustrate the points mentioned.

Hatching of Floating and Submerged Eggs

Fielding (1919) in Queensland, and Bacot (1916) in West Africa, found no difference in the hatching of floating and submerged eggs. In Manáos the difference was definite. Even under the most favourable conditions, floating eggs only occasionally hatched.

A batch of fifty-four eggs laid during the previous twenty-four hours were divided into two equal lots. Each lot was placed in a jar containing tap water (7·4 c.c. per egg), rice was added and the eggs were agitated by stirring daily for one minute. One lot was submerged on the first day, and the other left floating for twenty days. The results are shown in Table III.

Table III.

Hatching of Floating and Submerged Eggs.

Eggs floating for 20 days.	27	Eggs submerged on 1st day	27
Hatched by 20th day	0	Hatched by 20th day	77%
Hatched after submergence ...	96%	Hatched by 39th day	96%

This result has been confirmed by similar experiments with and without added food, and disturbance, and in rain water, but occasionally a few eggs hatched. Why some eggs hatched when floating, although the majority did not, is not apparent.

Under natural conditions the eggs were observed to be submerged by various agencies, chief of which appeared to be rain. In Manáos rain falls at all seasons.

Jars containing eggs were exposed to rain with the following results :—

41 eggs	out of	56	were submerged	by	15	minutes rain.
25 ,,	,,	26	,,	,,	5	,, ,,
18 ,,	,,	27	,,	,,	5	,, ,,

Eggs laid or becoming stranded on the sides of the receptacle became attached when dry, and when the water rose again remained attached and were therefore submerged. Fully developed larvae usually, but not always, submerged floating eggs, seizing the eggs with the mouth, pulling them below the surface and releasing them.

The following results were obtained :—

4 larvae	submerged	89	out of	110 eggs in 2 days.
4 ,,	,,	19	,,	remaining 21 eggs in 27 days.
6 ,,	,,	34	,,	34 eggs in 24 hours.
4 ,,	,,	4	,,	27 eggs in 11 days.

The reason for the differences shown is not known.

Floating eggs were also found to be submerged by insects falling into the water.

Effects of Disturbance on Hatching

Mitchell (1907), in the United States, records that Duprée found agitation to be a great factor in the hatching, and that if left undisturbed eggs may remain unhatched for over a year. Bacot (1916), in West Africa, failed to obtain a decisive result on this question of agitation, and also (1918) casts doubt on the value of Mitchell's records.

In Manáos the majority of eggs did not hatch unless disturbed. It has already been stated that floating eggs did not usually hatch, even when disturbed. When submerged before they were ready to hatch and left undisturbed, they also usually remained unhatched when no food was added. This is shown in Table IV:

TABLE IV.

Effects of Agitation on Hatching in the Absence of Added Food.

Nature of water		Submerged 1st day No food added: Not Agitated	Submerged 1st day No food added: Agitated	Control Food added: Agitated
Tap water	No. of eggs	50	30	17
	Hatched in 1 month	2%	10%	100%
Rain water	No. of eggs	140	30	25
	Hatched in 1 month	0	33%	96%

In this experiment two batches of eggs were used, one in tap water and the other in rain water. The control was merely to demonstrate that the eggs were fertile under favourable conditions. The agitation consisted of stirring for one minute daily. The amount of water per egg was the same for each batch.

In the following experiment shown in Table V, some hatching took place in the presence of added food, but less so than among the controls. Rice was added to each jar, and all eggs were submerged on the first day.

TABLE V.

Effects of Agitation on Hatching in the Presence of Added Food.

Water	230 c.c. in each jar	Not Agitated	Agitated
Rain water	No. of eggs	30	30
	Hatched in 12 days ...	40%	100%

It may be added that a further 36 per cent. hatched when agitation was provided.

Various methods of providing disturbance were tried. The dropping of water into the jar so as to submerge the eggs was usually followed, in the presence of food, by the hatching of the majority when they were four days old. Rain had a similar effect. A jar containing twenty-six eggs, four days old, floating in tap water, was placed in rain for five minutes. Twenty-five eggs were submerged, and within four hours eighteen larvae were removed. A control showed no hatching.

Stirring or aerating the water for one minute daily, or the addition of one or more larvae, provided an effective stimulus. In the presence of added food, the disturbance caused by larvae appeared to be only slightly more effective than one minute's stirring, as shown in Table VI.

TABLE VI.

Larvae *v.* Stirring in the Hatching of Eggs in Presence of Added Food.

Two fully-grown larvae present		Stirred one minute daily	
No. of eggs	30	No. of eggs	30
100% hatched in	5 days	100% hatched in	8 days

Each jar contained 230 c.c. of rain water. The eggs were submerged at the beginning of the experiment.

In tap water little difference was observed, the larvae hatching the eggs slightly faster. In the absence of added food, however, larvae were more effective, as shown in Table VII.

TABLE VII.

Larvae *v.* Stirring in the Hatching of Eggs in Absence of Added Food.

Two fully-grown larvae present		Stirred for one minute daily	
No. of eggs	30	No. of eggs	30
Hatched in 18 days	76%	Hatched in 18 days	10%
Total hatched after addition of rice	96%	Total hatched after addition of rice	90%

Each jar contained 230 c.c. of tap water. The eggs were submerged at the beginning of the experiment.

A similar result was obtained in rain water. In these experiments fully grown larvae which pupated or died were replaced by others. It seems probable from these and other results that the larvae, possibly through their excretions, had an effect on the eggs similar to that of the addition of food.

Effects of Food on Hatching

In Table VIII it is shown that the addition of rice rendered the conditions more favourable to hatching.

Table VIII.

Influence of Addition of Rice on Hatching.

Rice added	0	50 mgms
No. of eggs in each jar	30	30
Hatched by 20th day	33%	90%
Hatched after addition of rice	83%	—

The eggs were floating till submerged on the fourth day, after which they were stirred daily for one minute. Each jar contained 230 c.c. of rain water.

In other experiments where rice had not at first been used, but other conditions suitable to hatching were present, its addition was invariably followed by hatching, comparison with controls indicating that the hatching was due to the addition of the rice.

Effects of Differences in Water on Hatching

Tap water containing more organic matter than rain water might have been expected, on grounds of possible food supply, to be more suitable for the hatching of eggs than rain water. The latter was, however, found to be preferred by the mosquito for laying eggs on, and to be more suitable for the hatching and development of larvae. Tap water consists, in Manáos, of sedimented river water, an analysis of which, made by Mr. W. J. Debdin, F.I.C., F.C.S., has been published by Thomas (1910). According to this analysis the water contained a considerable amount of albumenoid ammonia, apparently derived from vegetable matter, no nitrates, but *B. coli* in 0·1 c.c., and in other ways, resembles what is usually described as a peaty water.

Where conditions were favourable for hatching, all, or nearly all, eggs hatched, whether in tap or rain water. In Table IX are shown the results of an experiment in which the conditions were favourable, there being 50 mgms. of rice in each jar; the eggs were submerged on the fourth day, and two larvae were added to each jar.

TABLE IX.

Hatching of Eggs in Different Waters under Favourable Conditions.

Nature of water (7 c.c. per larva)	Rain water	Tap water
No. of eggs in each jar	30	30
Hatched by 5th day	100 %	93 %

Where conditions were less favourable, eggs hatched more readily in rain water, as shown in Table X. Here no food and no larvae were added. The eggs were floating till submerged on the fourth day.

TABLE X.

Hatching of Eggs in Different Waters under less Favourable Conditions.

Nature of water (4·6 c.c. per larva)	Rain water	Tap water
No. of eggs in each jar	50	50
Hatched by 5th day	52 %	22 %

Similar results were obtained under other conditions, and it will be shown subsequently that larvae developed more quickly in rain water.

VIABILITY OF EGGS KEPT IN WATER

It is well known that eggs will hatch after being kept dry for many months. Bacot (1916) stated that some eggs when kept continually immersed did not hatch for periods of from two to five months. This was tested, and the results are recorded in Tables XI and XII. The eggs were stored in the water in jars, which were undisturbed as far as possible and to which no food was added. At the end of each month shown, a number of eggs were removed and examined, the split and collapsed ones being rejected and the others submerged in rain water to which rice was added and stirred daily.

TABLE XI.

Viability of Eggs stored in Tap Water.

	Removed after	No. tested	Hatched	Pupated	Adults
Eggs stored floating ...	4 months	20	45 %	0	0
	5 months	40	15 %	0	0
	6, 7, 8 months	160	0	0	0
Eggs stored submerged	4 months	30	46 %	6·6 %	6·6 %
	5 months	30	20 %	0	0
	6 and 7 months	30	0	0	0

TABLE XII.

Viability of Eggs Stored in Rain Water.

	Removed after	No. tested	Hatched	Pupated	Adults
Eggs Stored Floating	3 months	40	92 %	75 %	75 %
	5 months	30	40 %	...	... *
	7 months	25	0	0	0 *
Eggs stored submerged	3 months	40	100 %	95 %	95 %
	4 months	40	95 %	95 %	95 %
	5 months	32	56 %	...	... *
	7 months	15	0	0	0 *

* These observations were kindly made for me by Dr. R. M. Gordon after my departure from Manáos.

From each of the four batches used in the experiments shown in Tables XI and XII controls were taken and placed under conditions favourable to hatching, and were found to be fertile to the extent of 96 to 100 per cent. adults being eventually produced. Rejections on account of splitting or collapse of the eggs amounted to 7 to 11 per cent. of the eggs in each jar. Of the adults produced, sixty-four were males and forty-four females.

Comparison of the figures in Tables XI and XII would indicate that the eggs retained their viability longer in rain water than in tap water, but such a comparison is not justifiable as different batches of eggs were used, and the times of the experiments, although overlapping, were not identical.

Eggs were, therefore, found to be able to remain alive for five months in water, either floating or submerged. This accords with Bacot's findings in West Africa. Mitchell (1917) records survival immersed at over a year, but gives no details.

THE DEVELOPMENT OF LARVAE AND PUPAE

The development of *S. calopus* larvae is influenced by the nature of the water, its amount per larva, the presence of food and its nature, and other factors which were not investigated.

In each of the experiments shown in Tables XIII to XVI the larvae used were hatched from the same batches of eggs during the same respective periods, and were all less than twenty-four hours old at the beginning of the experiments.

Nature of the Water

The only waters compared were tap water and rain water. The result is shown in Table XIII. The larvae hatched in the water in which they were subsequently kept. 0·02 per cent. of rice was added to each jar, and the water was aerated daily for one minute by bubbling air through it.

Table XIII.

Development of Larvae in Tap Water and Rain Water.

Water (11 c.c. per larva)	Rain water	Tap water
No. of larvae	24	24
Pupation commenced	10th day	22nd day
Percentage giving pupae	79 %	8 %
Percentage giving adults	79 %	0
Average larval life of those pupating	19·9 days	22·5 days

In the tap water all the larvae became fully developed, but were undersized. The mortality was probably associated in some way with the water, but larvae were quite capable of developing in tap water when less crowded. Fourteen out of fifteen became adults under similar conditions in tap water where the concentration was 50 c.c. per larva.

Concentration of Larvae

The effects of overcrowding are shown in the following experiments, and indicate that where experiments are carried out to test the values of different foods or waters, the results are not comparable if the concentration of larvae has not been the same in each experiment.

Table XIV.

Result of Varying the Amount of Tap Water per Larva.

Amount of water per larva	50 c.c.	250 c.c.
No. of larvae in each jar	15	15
No. of pupae produced	15	15
No. of adults produced	14 (9♂♂; 5♀♀)	15 (10♂♂; 5♀♀)
Average duration of larval and pupal stages, ♂♂ ...	14·2 days	10·8 days
Average duration of larval and pupal stages, ♀♀ ...	17·8 days	13·0 days

This experiment is complicated by the fact that in the jar with 50 c.c. of water per larva there was only one-fifth of the quantity of rice present in the other jar (0·006 per cent.). As it became used up, therefore, rice was added gradually to the former jar till equal quantities had been placed in both without raising the percentage present at any time much above 0·006.

Table XV.

Variation of the Amount of Rain Water per Larva.

Amount of water per larva	15 c.c.	30 c.c.
No. of larvae	20	10
No. of pupae produced	19	10
No. of adults produced	19 (12 ♂♂; 7 ♀♀)	10 (5 ♂♂; 5 ♀♀)
Average duration of larval and pupal stages, ♂♂ ...	7·0 days	7·0 days
Average duration of larva and pupal stages, ♀♀ ...	8·3 days	7·4 days

Here the difference is less, possibly owing to more favourable conditions. It was again considered better to provide equal quantities of food per larva in each jar (0·025 per cent. rice and 0·006 per cent. peptone) by gradual addition rather than to commence with a double concentration of food in one jar. The water in each jar was aerated daily for one minute.

Assuming that the method of adding food did not introduce a fallacy, these and other experiments indicate that overcrowding may influence the rate of development.

Nature of Larval Food

A large number of organic substances have been found to be suitable as food for the larvae, but some appear to be more so than others. In the following experiment peptone and rice were compared. Two jars, each containing 400 c.c. of tap water, were taken, and rice was added to one and peptone to the other to the amount of 0·012 per cent. on the first and fourth days of the experiment. An equal number of eggs hatched in each jar during the same period of twenty-four hours. The water was aerated for one minute daily. Details are given in Table XVI.

Table XVI.

Peptone *v.* Rice as a Larval Food.

Food	Peptone	Rice
No. of larvae	19	19
No. of pupae produced	19	19
No. of adults produced	19 (15 ♂♂; 4 ♀♀)	18 (12 ♂♂; 6 ♀♀)
Average duration of larval and pupal stages, ♂♂ ...	7·1 days	8·6 days
Average duration of larval and pupal stages, ♀♀ ...	8·0 days	9·8 days

Thus under these conditions both male and female larvae develop more rapidly on peptone than rice.

Duration of Larval and Pupal Stages

The duration of the larval stages varied enormously under the conditions described above. The shortest time observed was four days in the case of three male larvae, the food used being peptone

(0·006 per cent.) and rice (0·025 per cent.) in rain water (30 c.c. to each larvae). The longest period recorded was also in the case of a male larva, which did not pupate till the forty-second day after hatching and became an adult two days later; in this case the food was rice alone, and the concentration 9 c.c. of rain water per larva. Macfie (1915) states that under 'normal conditions' the larval stage usually lasts seven to thirteen days, and records an instance where it lasted at least ninety-nine days and produced a healthy adult. Bacot (1916) states that under the most favourable conditions the larval life is passed within four days, but with scarcity of food is prolonged for upwards of seventy days.

Table XVII gives the average duration of a considerable number of larvae living under various artificial conditions in the laboratory.

TABLE XVII

Duration of Larval Stage.

Sex	No. of larvae	Average number of days	14 days and under
♂	77	9	90·9 %
♀	48	14·6	62·5 %
Unrecorded	57	7·3	92·9 %
Total	182	9·9	84·0 %

The duration of the pupal stage did not vary to any great extent. Figures are given in Table XVIII.

TABLE XVIII.

Duration of Pupal Stage.

Sex	No. of Pupae	1 day	2 days	3 days
♂	96	2	88	6
♀	62	2	53	7
Unrecorded	28	0	17	11
Total	186	4	158	24

Of these figures 85 per cent. took two days, and none took as long as four days. Macfie (1915), in West Africa, records the pupal stage as lasting one to five days, as does Mitchell (1907) in U.S.A.

Table XIX shows the duration of the combined larval and pupal stages.

Table XIX.

Duration of Combined Larval and Pupal Stages.

Sex	No. of Larvae	Average number of days	14 days and under
♂	105	10·3	85·7 %
♀	73	14·1	69·8 %
Total	178	11·6	79·2 %

As these records are based on observations made once daily only, and always about the same time, fractions of a day were not recorded. Individuals stated to have taken any particular number of days may be more or less than the number stated by just under twenty-four hours.

Survival of Pupae out of Water

Fielding (1919) placed five pupae on filter paper which was kept moist. All became adults within four days. Of three pupae placed on wet filter paper, allowed to dry and later removed to water, one hatched after thirty-two hours on the filter paper and two failed to hatch after forty-seven and a half and seventy-two hours. Alcock (1921) records that pupae left on the floor of a cage in London hatched after some delay.

In the following experiments the pupae were placed on blotting paper till dry and then transferred to dry glass tubes.

TABLE XX.

Survival of Dry Pupae.

Duration of pupal stage before drying	Under 24 hours	Over 24 hours, Under 48 hours
No. of pupae	12	3
Result	All died within 48 hours	All hatched within 6 hours

The dry bulb temperature varied between 29° and 25° C. and the wet bulb between 25° and 23° C. during this experiment.

When, however, two lots of dry pupae, four under and four above twenty-four hours, were kept in a dry tube, placed in a stoppered bottle containing water, three hatched out of each lot. It would, therefore, appear that the development of dry pupae was influenced by their age when dried, atmospheric moisture and probably other factors not investigated.

MISCELLANEOUS FACTS

Relative Numbers of Males and Females

The relative numbers of males and females bred in the laboratory are given in Table XXI.

TABLE XXI.

Relative Numbers of Males and Females.

Total Adults	No. of Males	Percentage of Total	No. of Females	Percentage of Total
517	323	62·5 %	194	37·5 %

This preponderance of males appeared to be a constant factor under the various conditions employed in the laboratory.

Removal of Eggs by Ants

As Bacot (1916) emphasises the fact that ants in West Africa did not carry off eggs, it is worth noting that in Manáos dry eggs were readily attacked by ants which were observed actually removing them, and batches of dry eggs left exposed on the bench overnight were frequently partly or wholly removed by the following morning.

Laying of Eggs without Feed of Blood

Fielding (1919) found that eggs were laid when peptone and sugar were given as food without blood. This was tried in Manáos without success, a thick syrup of sugar and peptone being supplied as food in a cage containing about fifty female *S. calopus* and a larger number of males during a period of one month. Eggs were laid when they were allowed to obtain blood. Mitchell (1907) states that *S. calopus* will mate and at times lay without feeding.

Oil as a Larvicide

Macfie (1917), discussing *S. fasciata* larvae imprisoned beneath a film of oil, describes the efforts made by the larvae to reach the surface. He writes as follows :—' So vigorous does the effort appear to be that it seems not improbable that it would eventually break through a thin film of oil.' The latter occurrence was observed in Manáos in the case of larvae breeding naturally in a barrel of water, the surface of which was covered with a thin film of oil. The larvae on ascending for air did not usually succeed in penetrating the surface film at the first attempt, but only after repeated efforts at different parts of the surface. The larvae from this barrel were the largest observed, but of some removed to the laboratory only a few reached the adult stage, the majority dying owing to failure to get clear of their larval or pupal skins. The pupae and adults which developed were also unusually large.

SUMMARY

Various points in the bionomics of *S. calopus* in Manáos have been investigated, and in certain minor respects they differed from those described in West Africa and Queensland. Bacot (1918), in discussing the duration of viability of the eggs of *S. fasciata*, writes, ' It seems to me possible that the African and American races of *S. fasciata*—to suggest no smaller division—may differ considerably in constitution.' It is, however, possible that the differences noted in various countries are due to differences in the conditions under which the experiments were carried out, and, therefore, conclusions cannot be drawn from their comparison.

The following facts were found to apply to *S. calopus* under the particular conditions described above :—

1. The adults laid more eggs in cesspit water than in river or tap water.

2. More eggs were laid on a damp surface than on the water surface.

3. Eggs did not usually hatch when floating.

4. Floating eggs were submerged by rain and *S. calopus* larvae.

5. Adults laid more eggs, eggs hatched earlier and in larger numbers, and larvae developed more rapidly and showed less mortality in rain water than in tap water.

6. Some eggs stored in water, floating and submerged, hatched after five months. None hatched after seven months.

7. Larvae developed more rapidly and showed less mortality with peptone as food than with rice.

8. The larval stage varied from four to forty-two days and the average duration in one hundred and eighty-two larvae was approximately ten days, the average for the females being longer than for the males.

10. The duration of the pupal stage varied from one to three days, 85 per cent. of one hundred and eighty-six pupae taking two days.

11. Pupae dried at least twenty-four hours after pupation developed into adults, although kept dry.

12. Throughout the experiments more males than females were produced.

13. Dry eggs were removed by ants.

14. No eggs were laid by adults fed on peptone and sugar only.

15. It was observed that larvae were capable of obtaining air through a thin film of surface oil.

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